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**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1669612> since 2021-12-23T12:50:21Z

*Published version:*

DOI:10.1002/rra.3294

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**Investigating the role of refuges and drift on the resilience of macroinvertebrate communities  
to drying conditions: an experiment in artificial streams**

Short running title: Resilience mechanisms of macroinvertebrates to droughts

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## **Acknowledgements**

The authors wish to thank the Servizio Bacini Montani, Autonomous Province of Trento, for building and maintaining the flumes. We are very grateful to Alfredo Maule and Francesco Macchi for their assistance and contribution during the field and laboratory activities. This work was realized within the framework of the PRIN NOACQUA “*Risposte di comuNità e processi ecOsistemici in corsi d'ACQUA soggetti o intermittenzo idrologico*” - codice 201572HW8F\_003, funded by the Italian Ministry of Education, University and Research.

## **Abstract**

Flow intermittence occurs in an increasing number of streams, due to climate change, local land-use alteration and water abstraction. In particular, droughts represent a new element in Alpine river regimes, and their ecological consequences are poorly explored. We here used artificial streams to investigate the resilience of macroinvertebrates to drought in Alpine streams based on the presence of pools (i.e., refuges) and drift (i.e., recolonization). Four flumes were selected: one with permanent flowing water (Control), while the other three (Drift+Pools, Only Drift, Incoming Drift) subjected to two consecutive drought-rewetting phases. The effects of droughts on benthic invertebrate communities and their recovery were assessed in terms of composition, structure, diversity and stability. Droughts dramatically reduced the biodiversity, especially with regard to the most sensitive and specialized macroinvertebrates, such as EPT, shredders and scrapers. Macroinvertebrate assemblages of the flumes that experienced drying phases were dominated by few generalist taxa and showed a higher degree of dissimilarity. Overall, no significant differences were observed in relation to the presence of pools, suggesting a limited role of this habitat in the recovery process. This finding seems to indicate that in shallow and fast-flowing Alpine lotic ecosystems the drift rather than pool availability represents the main driver of the macroinvertebrate resilience to droughts. Since the magnitude of droughts in Alpine streams and their frequency are expected to increase in the next decades due to the combined effects of local and global pressures, understanding which factors facilitate the recovery of aquatic communities assumes a fundamental importance.

**Key-words:** drought, Alpine streams, recovery, pools, drift, benthic invertebrates, outdoor flumes

## **1. Introduction**

An increasing number of lotic ecosystems are currently experiencing drying conditions and water shortage (Arthington, 2012; Humphries & Baldwin, 2003; Ledger & Milner, 2015). Although these phenomena are typical features of the natural flow regime in some areas, such as Mediterranean regions (Bonada, Rieradevall, & Prat, 2007b; Vannucchi, López-Rodríguez, de Figueroa, & Gaino, 2013), in the last decades they have been spreading out in other climatic regions where rivers are usually perennial. For example, in Northern Italy, Alpine streams are facing an intensification in both the magnitude and frequency of droughts due to the combined effects of global and local pressures (Chiogna et al., 2016; Fenoglio, Bo, Cucco, Mercalli, & Malacarne, 2010; McGregor, Petts, Gurnell, & Milner, 1995). Indeed, global warming is producing a raise in temperature regimes, a reduction in ice cover and a shift in the spatial and seasonal amount of precipitations (Beniston, 2012; Middelkoop et al., 2001). Moreover, these lotic ecosystems are increasingly impacted by heavy flow regulation and water abstraction. Therefore, Alpine streams are changing from perennial to intermittent systems with possible but still largely unknown detrimental ecological effects (Gorbach, Shoda, Burky, & Benbow, 2014; Leigh et al., 2016; Pinna et al., 2016). In this context, a great attention has been paid on the response of stream macroinvertebrates because their importance in terms of biomass, diversity and functionality (Allan & Castillo, 2007). The negative effects of droughts on benthic invertebrate communities briefly include: loss of biodiversity, especially with regard to the most sensitive species (Smith, McCormick, Covich, & Golladay, 2017; Storey, 2016), reduction in the organism abundance (Calapez, Elias, Almeida, & Feio, 2014; Řezníčková, Pařil, & Zahrádková, 2007) and changes in the functional composition and structure (Bonada, Doledec, & Statzner, 2007a; Ledger, Brown, Edwards, Milner, & Woodward, 2013; McKay & King, 2006). Little is actually known on which factors drive the resilience of benthic communities in perennial streams recently affected by droughts. The capacity of lotic systems to self-recover from disturbances mainly depends on their high heterogeneity and dynamicity over spatial and temporal scales and, last but not least, on the degree of natural integrity of biological communities at reach or basin scale (Boulton & Lake, 2008; Lake, 2000). In this context, beta-diversity (i.e., among sampling units) has been commonly used as an indicator of community stability and integrity in microbial ecology (Barberán & Casamayor, 2011; Zaneveld, McMinds, & Thurber, 2017), but similar approaches are available in stream ecology. For example, Gutiérrez-Cánovas, Millán, Velasco, Vaughan, & Ormerod (2013) evaluated patterns in beta-diversity between the macroinvertebrate communities affected by natural and anthropogenic

pressures, while Datry, Moya, Zubieta, & Oberdorff (2016) used this metric with regard to the hydrological regime, comparing assemblages from intermittent and perennial sites.

One of the most important factors to guarantee resilience of benthic invertebrate communities is the presence of in-stream refuges. A set of different micro-habitats have been suggested as refuge zones for macrobenthos during a period of water shortage: pools (Chester & Robson, 2011; Verdonschot, Oosten-Siedlecka, Braak, & Verdonschot, 2015), the hyporheic zone (Brunke & Gonser, 1997; Fenoglio, Bo, & Bosi, 2006; Otermin, Basaguren, & Pozo, 2002; Wood, Boulton, Little, & Stubbington, 2010), wet sediments, seeps, lateral aquatic habitats and the organic debris (Robson, Chester, & Austin, 2011). Recovery of invertebrate communities after droughts occurs not only by upward movements from subsurface (hyporheic) refuges, but also by downstream migration, notably by drift, and also hatching or reactivation of drought-resistant stages, upstream movements, and aerial re-colonization either by adults or through oviposition (Lake, 2000). However, drift represents the primary and fastest mechanism used by larvae of some aquatic insects to quickly recolonize vacant habitats (Fowler, 2004; Paltridge, Dostine, Humphrey, & Boulton, 1997; Robinson, Tockner, K., & Burgherr, 2004). Despite the great emphasis on the specific role played by each of these re-colonization sources, quantitative data are scarce and this is essentially due to the difficulties associated to data collection in field. Indeed, droughts are generally unpredictable: the inter-annual variability in the flow regime may result in differences in the magnitude and extent of the drying phase and this could interfere with the response of communities. In turn, this variability can affect the occurrence and availability of in-stream refuges within the same river section over the time and spatial scales. This highly variable situation makes difficult to compare the findings and draw general conclusions. In this context, the use of artificial streams and manipulative approaches can provide valid and replicable methods for overcoming the above-mentioned critical aspects (Bunn & Arthington, 2002; Lamberti & Steinman, 1993; Poff et al., 2003).

In this study we investigated the response of benthic invertebrate communities to drought in Alpine streams using outdoor artificial streams. Simulated droughts in artificial streams have been carried out in other studies (Lancaster & Ledger, 2015; Ledger, Edwards, Brown, Milner, & Woodward, 2011; Ledger, Harris, Armitage, & Milner, 2012), varying on average around 6 days in accordance with the size of the flumes. In particular, the aims of this work were: i) to assess the effects of drought on taxonomical and functional composition, structure, diversity and degree of stability of macroinvertebrate assemblages; ii) to evaluate the role of in-stream refuges (i.e., pools) and incoming drift on the recovery pattern of macroinvertebrate communities to short-term droughts. We hypothesized that drought strongly altered all the structural and compositional attributes of

benthic invertebrate communities and that pools partially buffered the detrimental effects of the drying phase, facilitating the resilience of macroinvertebrates.

## **2. Material and Methods**

### **2.1 Experimental design**

The study was performed in a set of outdoor artificial streams consisting of five 20-m long, 30-cm wide and 30-cm deep metal flumes (bottom surface area: 6.0 m<sup>2</sup>), directly fed by a second-order pristine Alpine stream (Fersina stream, Trento, NE-Italy). Invertebrates freely colonize the flumes by drift or bottom downstream movements. For more details on the flumes setting, see Bruno, Cashman, Maiolini, Biffi, & Zolezzi (2016).

For this study, four flumes were used, corresponding to different drought treatments (Figure 1): one maintained flowing water during all the experiment (Control: C), while the other three were subjected to drying phases. In one of the flumes, we artificially created pools using plastic buckets (39x28x14 cm). At the beginning of the experiment 16 plastic buckets were placed in the flume, flush with the bottom, and filled with cobbles (diameter: 8-10 cm) and fine gravel (diameter: 0.2-2 cm). This composition was the same as the substrate conditions within the artificial flumes during the whole experiment. When the buckets were submerged there were 7 cm from their upper edge and the surface of water, so that the artificial shape of the buckets did not hinder the water flow inside the flume. This flume represented the Drift+Pools (DP) treatment because the recovery pattern of benthic invertebrates was expected to be influenced by both factors. The second treatment flume (Drift Only: DO) was characterized by the absence of pools: it represented a condition where no refuges were provided to benthic invertebrates and their post-drought recovery was expected to depend exclusively on the drift from the upstream sections (i.e., from the river). We placed 16 standardized circular and drilled baskets (diameter: 22 cm) in each flume, filled with gravel and 6 cobbles of the same size of those in the flume. These baskets represented our sampling units for the macroinvertebrates. The third treatment flume (Incoming Drift: ID) had no pools nor baskets, and was used only to collect the incoming drift from upstream with a drift net (30 x 30 cm; 350 µm mesh size) placed at the water inflow. This flume was affected by the same dewatering and rewetting phases as the two treatment ones described above. The water velocity at the entrance of all the flumes was 0.4 m s<sup>-1</sup> and discharge 0.005 m<sup>3</sup> s<sup>-1</sup> and they were kept consistent during the rewetting phases for flumes DO, DP and ID, and throughout the entire experimental period in C. In summer 2016, two sets of simulations were performed to evaluate the effects and the recovery pattern of macroinvertebrate communities to short-term droughts.

We adopted a before-after-control-impacted (BACI: Smith, 2002) experimental design (Figure 2): after 21 days of natural colonization of the sampling units we collected benthic invertebrates from all the flumes. Then, we closed the gates of the DP, DO and ID flumes producing a 3 days-drought followed by 23 days of rewetting. At the end of this period we sampled the benthic invertebrates from all the flumes and we repeated this treatment twice, for a total of three sampling sessions. In this study three days of dewatering represented an acceptable trade-off between the need of producing drought conditions inside the flumes and prevent catastrophic alterations of the water characteristics inside the plastic buckets, guaranteeing their role as refuge. The dewatering period here applied is in accordance with other studies and it was effective to drain away the water from the flumes. During each sampling session, four randomly selected baskets from each flume were collected. All the content of the baskets was transferred in trays: cobbles were vigorously rinsed to remove all the invertebrates adhering to their surface. After each 3 days-drought event, samples of drifting invertebrates were collected from the net for the entire rewetting phase and the drift was expressed as N. ind. m<sup>-3</sup>. The benthic and drift samples were filtered through a 350-um mesh and the invertebrates preserved in 70% ethanol solution. In the laboratory, macroinvertebrates were, sorted, counted, and identified to genus for Ephemeroptera and Plecoptera and family for the other groups following Campaioli, Ghetti, Minelli, & Ruffo (1994; 1999). In addition, taxa were classified into Functional Feeding Groups (Merritt, Cummins, & Berg, 2008).

## **2.2 Statistical analysis**

Statistical analyses were performed in the R environment (R Development Core Team, 2015), using the “vegan” package (Oksanen et al., 2015). We first visually evaluated the differences in the composition and structure of benthic invertebrate communities with a Non-metric Multidimensional Scaling (NMDS), performed with a Bray-Curtis similarity matrix. A Permutational Analysis of Variance (PERMANOVA) was used to test significant effects of the “drought treatment” (i.e. comparing C, DO and DP), “sampling session” (i.e. Before, Drought1 and Drought2), as well as their interaction on the benthic community composition. In a second step, we tested the effects of different treatments on the stability of the assemblages, by evaluating the dissimilarity among sampling units within each treatment, i.e. beta-diversity. Following the procedure proposed by Gardener (2014), we used the Bray-Curtis index as a metric of similarity between the samples and this analysis was performed using presence/absence data and the R function “vegdist”. The differences in the beta-diversity, measured with the “betadisper” function (Anderson, 2006) as distance from the median in relation to the drought treatment and sampling sessions were visually assessed with a Principal Coordinate Analysis (PCoA). We tested the effects of treatments, sessions



and their interaction on beta-diversity, benthic invertebrate biodiversity and Functional Feeding Groups (FFGs), expressed as differences in the total taxa richness, EPT richness and evenness index by running a two-way Analysis of Variance (ANOVA). For this analysis, count data were  $\log(x+1)$  transformed and proportion data of all FFGs were arcsin-transformed to achieve the normal distribution of residuals. The drought treatment and the sampling session variables were used as fixed factors. The Tukey's HSD test was used for the post-hoc comparisons. Among biodiversity metrics, besides the total taxa richness, we focused on the EPT taxa richness because they are a key component of benthic assemblages in Alpine streams and also the most sensitive taxa. In addition, the evenness index (i.e. 1-Simpson's dominance index) for each sample was calculated to further investigate the consequences of the dewatering and rewetting on the structure of macroinvertebrate communities. Moreover, to evaluate the different contribution of each flume to the total diversity, we calculated the ratio between the total number of taxa for each flume in each sampling occasion (i.e. pooling together the four samples) divided for the total number of taxa recorded in this study. This operation allowed us to evaluate the cumulative effects of pools and drift on the dwelling invertebrate communities and to estimate the loss of biodiversity due to the droughts. Finally, drift data collected in the additional flume (ID) were used to draw a taxa accumulation curve to evaluate the temporal contribution of drift to richness during the rewetting phase. The samples of the two rewetting phases were pooled together into a unique database for this purpose. Moreover, the mean drift density of each taxon was calculated to account for the taxon-specific response.

### **3. Results**

The NMDS ordination (2 axes, stress = 0.19) showed that drought events caused marked changes in the composition and structure of benthic invertebrate communities (Figure 3). In particular, the results of the PERMANOVA illustrated significant effects of the drought treatment, the sampling session and also their interaction on community composition (Table 1). Before the drought events, the composition of the benthic communities in all the artificial flumes was very similar, confirming that the colonization period was adequate to exclude any confounding effect and provide comparable conditions among the different drought treatments. After the two consecutive short-term droughts only the Control flume (C) maintained a composition and structure of benthic invertebrate community similar to that observed at the Before session. On the contrary, the macroinvertebrate assemblages in the DO and DP flumes at the end of the simulations differed markedly from the initial ones.

The similarity among sampling units was used to test the stability of the benthic assemblages among the experimental conditions, and we observed significant differences both for drought

treatment ( $P < 0.01$ ) and sampling session ( $P < 0.01$ ). When the treatments were compared with a PCoA (Figure 4a), DP and DO showed a significantly higher dissimilarity than the Control. This result indicated that the degree of similarity among samples in the Control was high throughout the experiment, while in the flumes affected by the drought the dissimilarity markedly increased. However, the post-hoc comparisons showed significant differences only between the C and DP ( $P < 0.01$ ), while no significant differences were observed between the C and DO, although the  $P$ -value was close to the significant threshold ( $P = 0.059$ ). When the variation in beta-diversity among sampling sessions was analyzed with the PCoA (Figure 4b), we detected significant differences between the Before samples and those collected in the two consecutive sampling dates. In other words, before the droughts benthic communities had a high level of similarity regardless of the flume, whereas in the two consecutive sampling occasions the degree of dissimilarity between Control and treated flumes increased remarkably ( $P < 0.01$ ).

The results of the two-way ANOVA showed significant effects of the drought treatment, the sampling session and also their interaction on taxa richness (Table 1). Before the droughts, all the flumes showed the highest and comparable values of taxa richness (Figure 5a), ranging from 13 to 18 taxa (mean = 15.5, SD = 1.4). Conversely, after both the dewatering and rewetting phases, the richness dropped significantly in the DO (mean = 9.0, SD = 0.8) and DP (mean = 9.6, SD = 2.7) flumes in comparison to the Control (mean = 15.6, SD = 1.5). No statistical differences in the total taxa richness were observed between the DO and DP with regard to the post-drought sampling sessions. Similar results were obtained by the analysis of Ephemeroptera, Plecoptera and Trichoptera richness was analyzed (Figure 5b). The EPT richness was significantly affected by the treatment, the session and their interactions (Table 1). Before the drying phases, high and comparable values of EPT richness, varying from 8 to 11 taxa (mean = 9.5, SD = 0.9), were recorded in all flumes. Droughts significantly reduced the EPT richness in the DO (mean = 3.8, SD = 0.5) and DP (mean = 4.6, SD = 1.2), while the Control maintained values similar to the initial ones (mean = 8.5, SD = 1.3). We did not find statistical differences in the number of EPT taxa between the DO and DP flumes in both the post-drought sampling sessions.

Analogous results were obtained in terms of relative richness (Figure 5c): before the drought events the C, DO and DP flumes accounted respectively for 0.70, 0.76 and 0.66 of the total richness collected in this study. Again, these values markedly dropped in the flumes subjected to the drying phase compared to the Control, but this reduction was different in the two flumes. In presence of pools (DP) the proportion of taxa remained relatively high after the first drought event (% richness = 0.63 for Drought1), and it decreased to 0.46 after the second event (Drought2). By contrast, in the

flume characterized by the absence of pools (DO) the % richness after the two drought events was respectively 0.43 and 0.46.

Droughts affected also the evenness of benthic invertebrate communities (Figure 5d). Results of the ANOVA showed a significant effects of drought treatment, while no significant differences were detected in relation to the session and their interaction (Table 1). In general, drought reduced the evenness within the benthic invertebrate communities, facilitating the dominance of few generalist taxa. *Baetis* sp., Chironomidae and Simuliidae represented the most dominant macroinvertebrates in the present experiment: they together constituted an average of 80% of the total abundance before the drying phases. However, after the drought events, their relative abundance increased over the 95% both in the DO and DP flumes and no statistical differences were observed in the evenness between these two treatments. This trend, instead, was not recorded in the Control flume for which the relative abundance of these taxa remained similar to the initial one.

With the exception of filterers, we found significant results of the droughts on the functional composition of benthic invertebrate (Figure 6), with particularly evident impacts on scrapers and shredders, for which we recorded an effect of sampling session and the interaction between this factor and the drought treatment (Table 1). Scrapers instead were significantly affected only by this latter factor. Before droughts, the proportion of scrapers and shredders was on average 6.6% and 2.4%, respectively. The percentage of abundance of scrapers in the flumes affected by the drying phases fell to 0.9% (DO) and 1.3% (DP) after the first drought and 0.6% (DO) and 0.3% (DP) after the second drought. A similar trend was observed for shredders: their initial percentage abundance was on average 2.4%, and after the droughts it collapsed to 0% (Drought1) and 0.3% (Drought2) in the DO, and 0.2% and 0% in the DP. Elmidae, Heptageniidae and *Ancylus* sp. represented the most abundant scrapers, while the most common taxa among shredders were *Leuctra* sp. and *Sericostoma* sp. Results of the ANOVA showed a significant effect of the session on the percentage abundance of predators (Table 1), while we did not find any effect of the drought treatment or their interaction on this FFG. On average, the initial proportion of predators was 1.9% and then it was reduced to 0.5% (DO) and 0.75% (DP) after the two consecutive short-term droughts respectively. Large stoneflies, such as *Isoperla* sp. and *Dinocras* sp., and Rhyacophilidae represented the main predator taxa. The percentage abundance of collector-gatherers was statistically affected only by the drought treatment (Table 1). This FFG was the most abundant throughout all the experiment: several taxa were grouped in this category but *Baetis* sp., *Serratella* sp. and Chironomidae were the most abundant. However, the proportion of collector-gatherers was slightly higher in the DO and PD compared to the control. No significant differences in the percentage abundance of filter-feeders were observed. This group was represented by two families: Simuliidae and Hydropsychidae.

The majority of macroinvertebrate taxa quickly re-colonized the artificial flumes by drift (Figure 7a). The accumulation curve illustrates that few days of rewetting were effective to rapidly increase the number of drifting taxa, enhancing the total richness in the flume. However, we found that the drift density varied markedly among the macroinvertebrates (Figure 7b), with *Baetis* sp. showing the highest values ( $0.0042 \pm 0.0009$  ind.  $m^{-3}$  mean  $\pm$  SE), followed by Simuliidae ( $0.0018 \pm 0.0004$  ind.  $m^{-3}$ ) and Chironomidae ( $0.0014 \pm 0.0003$  ind.  $m^{-3}$ ). The large majority of taxa showed drift rates less than 0.0001 individuals  $m^{-3}$ .

#### 4. Discussion

The occurrence and intensification of flow intermittency in naturally perennial rivers is a recent phenomenon (Ledger & Milner, 2015). Notwithstanding a growing number of studies, the consequences of this alteration on the diversity and survival of aquatic biota are still poorly explored. The objective of this experimental study was to examine the response of benthic invertebrate communities to repeated, short-term summer droughts in Alpine streams using artificial streams, and the importance of instream refuges (i.e. pools) versus incoming drift in determining post-drought recovery. Our findings demonstrate that droughts strongly alter the composition and structure of benthic invertebrate communities, with negative impacts on all the descriptors of biodiversity. When the beta-diversity was calculated to assess the stability of the invertebrate communities, we observed that droughts increased the dissimilarity among the samples. Despite the initial colonization period and the rewetting phases consisted in a very comparable amount of time (21 and 23 days respectively), we found a higher dissimilarity among the post-drought communities in relation to the Control ones or those collected before the simulations. This result suggests that the re-colonization of macroinvertebrates in the flumes affected by droughts probably needs a longer time to reach a stable composition, comparable to the pre-drought levels. Moreover, macroinvertebrate assemblages affected by drying phases showed a marked reduction in taxa richness. This depletion in biodiversity was mainly driven by the loss of the EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa, which are typically the dominant groups in the Alpine streams as well as the most sensitive taxa. While few other studies employed artificial flumes to study the ecological impact of droughts (Lancaster & Ledger, 2015), our work is probably the first to assess in artificial conditions the relative importance of refuges and pool in post-drought recovery process. We found that the benthic invertebrate communities in the flumes affected by droughts were dominated by few generalist taxa, such as *Baetis* sp., Chironomidae and Simuliidae. These taxa have been associated to the early stages of the recovery from several sources of impact, including droughts (Otermin et al., 2002; Ledger et al., 2011).

Benthic invertebrate communities facing drying conditions appear impoverished, especially in relation to their key faunal components, altered and characterized by an unstable composition. Therefore, they may be more susceptible and vulnerable to the effects of other concomitant stressors. Taxonomical changes are often coupled with functional ones and impacts of droughts on the functional composition and structure of benthic communities have been demonstrated by several authors (Acuña et al., 2005; Bogan & Lytle, 2007; Fenoglio et al., 2007; Maamri, Chergui, & Pattee, 2005). According to these works, in the present study we recorded a significant decline of the proportion of scrapers and shredders in the communities experiencing the drying phases, with consequent repercussions on the other biological components of the food webs.

One of the main aims of this work was to evaluate the role of pools and drift in the post-drought recovery of macroinvertebrate communities. Conversely, to our hypothesis, we did not find significant differences between the flumes with incoming drift and with or without instream refuges (pools) for all the diversity metrics. Nevertheless, it is interesting to note that when the richness was calculated in terms of percentage of taxa, the loss of biodiversity after the first drought event was lower in presence of pools than in their absence. However, after the second drought event we did not detect statistical differences between these two flumes. These findings seem to indicate that pools in shallow and fast-flowing systems like Alpine streams may buffer the effects of water scarcity, acting as a refuge for benthic invertebrates only in relation to occasional droughts. On the contrary, repeated droughts seem to offset their contribution in the recovery pattern. These results are in disagreement with the evidences provided by other authors: for example Vander Vorste, Malard, & Datry (2015) observed that the post-drought recovery of macroinvertebrates was mainly accounted for other refuges, such as the hyporheic zone, rather than the drift. A manipulative approach was also adopted by Verdonshot et al., (2015) who observed that pools represented the main refuge habitat for benthic invertebrates. However, both these studies were carried out in a lowland and alluvial river, thereby different from the mountain stream here considered. By contrast, Fowler (2004) investigated the post-drought recovery of benthic invertebrate communities in two New Zealand streams, comparing down-stream intermittent sites with up-stream perennial ones. The author observed a very quick response: 95% of the macroinvertebrate taxa recolonized the sites within 7 days, indicating a substantial contribution of drift for the up-stream sections. Our results on drift seem to corroborate the latter hypothesis: we generally found a quick re-colonization by drifting taxa. However, drift rates varied largely among taxa and such differences could account for the dominance and evenness recorded in the post-drought communities. Based on these results, drift seems to be the main factor driving the post-drought recovery of benthic invertebrate communities in our study system.

Like all the experiments carried out in artificial streams, our study suffers some limitations due to the experimental conditions (Lamberti & Steinman, 1993). These include for example the attributes of the pools here used (i.e. plastic buckets isolated from the bottom) and the characteristics of the simulated drought-rewetting treatments. We are aware of this and further studies are needed to validate our results, involving the adoption of field surveys and manipulative approaches. However, artificial flumes have been successfully used and recommended in scientific literature for investigating the response of benthic organisms to several abiotic conditions, especially for those being difficult to isolate and quantify in the field (Bunn & Arthington, 2002; Lancaster & Ledger, 2015; Ledger et al., 2011; 2012; Poff et al., 2003;).

In conclusion, our experiment provides quantitative data on the impacts of droughts on Alpine macroinvertebrate communities and sheds light on the role of pools and drift in the re-colonization dynamics. Since the magnitude of droughts in Alpine streams and their frequency are expected to increase in the next decades due to the combined effects of local and global pressures, understanding which factors facilitate the recovery of aquatic communities assumes a fundamental importance.

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## Tables

Table 1. statistics (*F*- and *P*-values) for (a) PERMANOVA and two-way ANOVAs for (b) diversity metrics and (c) Functional Feeding Groups (FFGs) in relation to the drought treatment, sampling session and their interaction.

	Drought treatment		Sampling session		Interaction	
	<i>F</i> <sub>2,26</sub>	<i>P</i>	<i>F</i> <sub>2,26</sub>	<i>P</i>	<i>F</i> <sub>4,26</sub>	<i>P</i>
<b>PERMANOVA</b>	4.809	< 0.01	12.25	< 0.01	2.242	< 0.01
<b>Diversity metrics</b>						
Taxa richness	19.116	< 0.001	17.668	< 0.001	5.679	< 0.01
EPT richness	37.937	< 0.001	53.882	< 0.001	7.393	< 0.001
Evenness index	6.811	< 0.01	1.532	> 0.05	0.798	> 0.05
<b>FFGs</b>						
Scrapers (Sc)	37.403	< 0.001	8.716	< 0.01	15.329	< 0.001
Shredders (Sh)	0.606	> 0.05	5.117	< 0.05	4.661	< 0.01
Predators (P)	0.459	> 0.05	5.499	< 0.05	1.850	> 0.05
Collector-gatherers (Cg)	4.195	< 0.05	0.688	> 0.05	0.318	> 0.05
Filterers (F)	1.127	> 0.05	2.058	> 0.05	0.693	> 0.05



## Figure legends

Figure 1. Representation of the experimental design: letters indicate the drought treatment: C (Control), DO (Drift Only), DP (Drift+Pools) and ID (Incoming Drift). Circles with the ellipses inside represent the sampling units (i.e. drilled baskets filled with cobbles), while the blue areas indicate the flowing water in the C flume and the pools (i.e. plastic buckets) in the DP flume. The grey area in the ID flume indicate the drift net placed at the sluice gate.

Figure 2. Experimental design: the black line represents flowing water inside the flumes, dashed line represent the 3-days of drought. Sampling sessions were performed before starting the simulations, and after each drought-rewetting phases.

Figure 3. Ordination of the sampled macroinvertebrate communities according to the first two NMDS axes. Colors indicate the drought treatments: Control (C, black), Drift Only (DO, dark grey) and Drift+Pools (DP, light grey); symbols indicate the sampling sessions: Before (circle), Drought1 (square) and Drought2 (triangle).

Figure 4. Principal Coordinate Analysis (PCoA) ordination plots based on the dissimilarity matrices (Bray-Curtis). In each plot, symbols represent the benthic community samples according to the: (a) drought treatment (C = Control; DO = Drift Only; DP = Drift+Pools) and (b) sampling session (B = Before; Dr1 = Drought1; Dr2 = Drought2). The lines link each sample with its corresponding centroid.

Figure 5. Boxplots represent the variation in: (a) all invertebrate taxa richness, (b) EPT (Ephemeroptera, Plecoptera and Trichoptera) richness; (d) evenness for the different drought treatment and sampling session. Black line: median value; box: quartile interval; whiskers: minimum and maximum values. Bars indicate the percentage of taxa recorded in each flume after each session (c).

Figure 6. Stacked bars illustrate the percentage abundance of the Functional Feeding Groups (Sh = shredders, Sc = scrapers, P = predators, F = filterers, Cg = collector-gatherers) in each flume (C = Control; DO = Drift Only; DP = Drift+Pools) for each sampling session (Before, Drought1, Drought2).

Figure 7. Taxa accumulation curve calculated on the drift samples (a): the black line represents the mean number of taxa in each sample, while the grey area indicates the 95% confidence interval. The drift density of the benthic invertebrate taxa (b): the grey bars represent the mean values (+SE).